DATA EVALUATION RECORD
FORAGING ACTIVITY AND MORTALITY- HONEY BEES AND BUMBLEBEES
Apis mellifera* and *Bombus terrestris
(NON-GUIDELINE STUDY)

1. **CHEMICAL:** Imidacloprid PC Code No.: 129099

2. **TEST MATERIAL:** Imidacloprid® WG 70 Purity: 70%

3. **CITATION**

Authors: Ch. Maus, R. Schoening and J. Doering

Title: Assessment of effects of Imidacloprid® WG 70 on foraging activity and mortality of honey bees and bumblebees after drenching application under field conditions on shrubs of the species *Rhododendron catawbiense grandifolium* surrounded by other ornamental plant species

Study Completion Date: February 8, 2006

Laboratory: Bayer CropScience AG, Institute for Ecotoxicology, D-40789 Monhein, Germany

Sponsor: Bayer CropScience AG, Environmental Science, Lyon, France

Laboratory Report ID: G201808

MRID No.: 473034-05

DP Barcode: D348269

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental Inc.
Signature: **Date:** 05/28/08

5. **APPROVED BY:** Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.
Signature: **Date:** 06/04/08

6. **APPROVED BY:** Joseph DeCant, Ecologist, EPA/OPP/ERB V
Signature: **Date:** 07/01/14

7. STUDY PARAMETERS

Test Species: *Apis mellifera* and *Bombus terrestris*

Age of Test Organisms at Test Initiation: Not specified for bees. *Rhododendron* shrubs were approximately 7 years old and the other ornamental plants ranged in age from 8 weeks to 12 months

Test Duration: Bees were observed on 11 different days, blossom samples were collected from *Rhododendron* shrubs 126 days following application

8. CONCLUSIONS:

Rhododendron plants received a soil drench treatment 126 days before the start of the study with Imidacloprid® WG 70 at either 4.3 g ai/m plant size (2.58 g ai/shrub) or 2.15 g ai/m plant size (1.29 g/shrub), resulting in blossom residues of up to 1.996 mg imidacloprid/kg or 0.812 mg imidacloprid/kg, respectively. These plants were surrounded in a field by a species composition of ornamental plants. No mortality or behavioral abnormalities were observed for foraging bumblebees and honey bees. Bumblebee foraging activity was scarce on the ornamental plants and greater in the untreated *Rhododendron* plants compared to the treated ones by nearly a factor of two. Honey bee foraging was also scarce on the ornamental plants, and only one bee was observed foraging on an untreated *Rhododendron* plant; no honey bees foraged on the treated *Rhododendron* plants.

The study is limited by the fact that the honey bees and likely the bumblebees as well had alternate sources of pollen and nectar than the treated and control plots. Given the methods used to determine bee mortality, the absence of observed mortality cannot be construed as the absence of mortality. Furthermore, there is uncertainty as to the extent that 126 DAT represents the highest residue levels. Residue samples were taken at only one time point after treatment, and therefore time trends in the residue levels were not quantified.

9. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: This is a non-guideline study that did not adequately control for honey bee and bumble bee foraging activity. Additionally, imidacloprid residues were detected in control samples.

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: This is a non-guideline test.

11. SUBMISSION PURPOSE: This study was conducted to determine the effects of a drenching application with Imidacloprid® WG 70 to shrubs of the species *Rhododendron catawbiense grandifolium*, surrounded by an ornamental species composition typically found in suburban gardens in North America, on foraging activity and mortality of honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) under field conditions. Additionally, the imidacloprid residues and those of its hydroxy- and olefin-degradates in blossom samples of the *Rhododendron catawbiense grandifolium* shrubs were measured in order to determine exposure to pollinators.

12. MATERIALS AND METHODS

A. Test Organisms

Shrubs of *Rhododendron catawbiense grandifolium* (approx. 7 years old) were located at the experimental farmland "Laacher Hof" near Monheim (40789 Monheim, Nordrhein-Westfalen, Germany). Shrubs were planted in sets of 10 plants arranged in line (equaling 1 replicate), distributed in 5 parallel rows on 1 plot. For this study, only 3 of the 5 rows were used. The distance between shrubs within a set was 1 m and between the sets within one row 2 m. The distance between rows was 2.5 m. Each plot was used for one treatment group. Shrub height and width averaged 0.6 m. Bee-attractive potted ornamentals in watering trays were set up between the rows of *Rhododendron catawbiense grandifolium* shrubs. The ornamentals were *Fuchsia* sp. (variety "Beacon"), strawberry plants (variety "Fragoo"), *Alyssum* sp., *Lantana camara* and *Lobelia* sp. The plants were evenly distributed among the three plots, such that each plot received 336 *Lobelia* sp. and 44 of each of the other ornamental plants.

One honey bee hive containing 20,000-25,000 honey bees and a queen and 3 bumblebee colonies containing 100-150 bumblebees each were set up next to the three plots. The honey bee hive was 5 m away from the 4.3 g ai/m plant height plot, 15 m away from the 2.15 g ai/m plant height plot and 35 m away from the control plot. One bumblebee colony was placed 5 m away from each of the three plots.

B. Test Design

Three treatments were included in the design: a negative control receiving no treatment; one group received 4.3 g ai/m plant height (2.58 g ai/shrub); and one group received 2.15 g ai/m plant height (1.29 g ai/shru). Each treatment was replicated three times, but all replicates were at the same

location. The water application rate was 1 L tap water per plant.

Appropriate amounts of test solutions were prepared by mixing calculated amounts of the test material with tap water, such that each plant would receive one liter of test solution. Control plants received no treatment. The solutions were applied directly onto the soil near the stems of the plants. Applications were made on January 13, 2005. The honeybee colony was set up on May 20, 2005 and the bumblebee colonies were set up on May 21, 2005.

Foraging activity was assessed for a total of 10 days in the morning and afternoon. Assessments were made from May 21-25, 2005, on May 27, 2005 and from May 30 to June 2, 2005. Foraging was determined by counting the number of honey bees and bumble bees on the *Rhododendron* plants and in the ornamental rows. Mortality was determined daily by counting the number of dead bees found on linen sheets placed along the ground in between plants. Mortality was also determined by counting the number of dead bees on linen sheets plance in front of the honey bee hive and the bumble bee colonies.

Samples of the *Rhododendron* blossoms were analyzed for residues of imidacloprid and its olefin- and hydroxy-degradates. Samples were analyzed using HPLC-MS/MS. The LOQ and LOD were 0.005 and 0.0015 mg ai/kg, respectively, for imidacloprid and its hydroxy-degradate and 0.010 and 0.003 mg ai/kg, respectively, for the olefin-degradate. Only replicate A was analyzed from the control on study day 17 since only samples from that replicate were taken. Both replicates from all other pre-flowering treatments were analyzed on both sampling days.

13. REPORTED RESULTS

Table 1. Range of residues (mg ai/kg) of imidacloprind and its hydroxy and olefin degradates in rhododendurn blossoms 126 days after treatment (DAT) with Imidacloprid® WG 70 to soil at base of plants.

Treatment Group	DAT	Imidacloprid mg ai/kg	Hydroxy-Imidacloprid mg ai/kg	Olefin-Imidacloprid mg ai/kg
Control	126	<LOQ – 0.037	<LOQ – 0.005	<LOQ – 0.001
4.3 g ai/m plant size	126	0.488-1.996	0.073-0.215	<LOQ-0.027
2.15 g ai/m plant size	126	0.092-0.811	0.014-0.060	<LOQ-0.012

Imidacloprid and hydroxy-degradate: LOQ- 0.005 mg ai/kg, LOD- 0.0015 mg ai/kg
Olefin-degradate: LOQ- 0.010 mg ai/kg, LOD- 0.003 mg ai/kg

Table 2. Morning (am) and afternoon (pm) total foraging activity in number of bumble bees (BB) and honey bees (HB) in vicinity of rhododendrens and ornamental plants.

Treatment Group	Rhododendron am		Ornamentals am		Rhododendron pm		Ornamentals pm	
	BB	HB	BB	HB	BB	HB	BB	HB
Control	120	0	3 (Fuchsia)	0	126	1	2 (Fuchsia)	2 (strawberry, *Lobelia* sp.)
2.15 g ai/m plant size	72	0	1 (Fuchsia)	0	59	0	2 (Fuchsia)	1 (strawberry)
4.3 g ai/m plant size	70	0	0	0	63	0	0	1 (*Lobelia* sp.)

BB- Bumblebees
HB- Honeybees
am- Morning
pm- Afternoon

No dead honey bees or bumblebees were observed throughout the study and no behavioral abnormalities were noticed. Foraging activity of bumblebees on the *Rhododendron* plants was similar between the morning and afternoon assessments with foraging activity greatest in the control group (**Table 1**). However, untreated *Rhododendron* plants were visited more than the treated ones. *Fuchsia* plants were scarcely visited by bumblebees; no other ornamentals were visited by bumblebees.

Only one honeybee was observed foraging on a *Rhododendron* plant, with the one observation occurring in the control plot. Honey bees were observed foraging on strawberry and *Lobelia* sp. plants; no other ornamentals within the study were visited (**Table 1**). The beekeeper noticed that honey bees returning to the hive carried yellow pollen, which probably originated from plants other than the ornamentals set up in this study.

No residues were detected in the controls. In the treated shrubs, residues of the parent material were found in blossoms up to a concentration of 1.996 mg imidacloprid/kg (**Table 2**).

14. REVIEWER COMMENTS:

The study does not describe the extent to which alternate food pollen/nectar sources may have been available. Foraging data indicate that honeybees did not appear to forage in the rhododendrons and only made very limited use of the other ornamental plants provided. Since honeybees were observed by the beekeeper to have pollen in their collection baskets, this implies that the bees were foraging in some other area than the treated site.

Storage stability tests were not reported so it is uncertain whether any effort was made to document the stability of the imidacloprid residues under the study conditions.

Foraging data (**Table 1**) on bumble bees suggest that they preferred untreated rhododendrons with roughly half as many bees foraging on treated rhododendrons. Similar to honeybees, the bumble bees (**Table 1**) were not attracted much to the other ornamental plants provided.

The study did not determine the extent to which dead bees could be identified on the linen sheets used to collect this information. Typically, studies examining bee mortality rely on drop zone dead bee traps that have screening to reduce dead bee removal by scavengers. The absence of any observed bee mortality in this study therefore cannot be construed as the absence of mortality.

The fact that bees took advantage of alternate food sources other than the treated area limits the utility of this study in estimating the potential.

In one of the control plants, imidicloprid residues were detected along with its two degradates. Imidacloprid residues in this control were roughly seven time the limit of quantitation. Maximum residues of imidacloprid in rhododendron blossoms were 0.811 and 1.996 mg/kg in the 2.15 and 4.3 g/m treatments, respectively (**Table 2**), 126 DAT. There is uncertainty as to the extent that 126 DAT represents the highest residue levels. Residue samples were taken at only one time point after treatment, and therefore time trends in the residue levels were not quantified.

15. REFERENCES:

No references were provided.